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14. ABSTRACT The overall goal of the VISION project is to discover neuroprotective strategies in three separate mouse models of injury to the visual axis, in order to identify potential candidates for the treatment of combat eye injuries and preserve vision in our injured warfighters. We have established three different mouse models of ocular injury with different injury-initiating mechanisms (i.e. optic nerve crush, retinal ischemia/reperfusion, and chronic ocular hypertension). We have developed techniques to quantify damage to the retina, optic nerve, and visual axis in the brain (i.e. superior colliculus) that are damaged in these three models. We are testing neuroprotective agents and strategies, including neuroprotective estrogens, sigma-1 agonists, Brn3b, inhibitors of Jun N-terminal kinase (JNK), and inhibitors of protein stress to determine their efficacy in protecting the retina, optic nerve and superior colliculus from the damage induced by each of the 3 models.					
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VISION Grant Year 4 (2013) Annual Report

INTRODUCTION

This is the fourth year annual report for the VISION (Vision Integrating Strategies in Ophthalmology and Neurochemistry) project at UNTHSC. Although we only received funding for the first two years of this ambitious 5 year project, we have continued working on the overall objectives of this project through judicious use of our remaining resources and no-cost extension requests. We currently have 3 PIs, 2 postdoctoral fellows, 5 graduate students, and 3 research technicians actively involved in this very project. In combat situations, traumatic eye injuries are frequent, leading to irreversible damage to the visual axis. The overall goal of the VISION project is to discover neuroprotective strategies in three separate mouse models of injury to the visual axis, in order to identify potential candidates for the treatment of combat eye injuries and preserve vision in our injured warfighters. We have established three different mouse models of ocular injury with different injury-initiating mechanisms (i.e. optic nerve crush, retinal ischemia/reperfusion, and chronic ocular hypertension). We have developed techniques to quantify damage to the retina, optic nerve, and visual axis in the brain (i.e. superior colliculus) that are damaged in these three models. We are testing neuroprotective agents and strategies, including: sigma-1 agonists, Brn3b, inhibitors of Jun N-terminal kinase (JNK), inactivation of C1q, NRN1, and inhibitors of protein stress to determine their efficacy in protecting the retina, optic nerve, and superior colliculus from the damage induced by each of the 3 models. In addition, we are also evaluating time dependent, injury-induced changes in gene expression in the effected tissues to identify the major pathogenic pathways involved in order to develop new therapeutic approaches for neuroprotection and neuroregeneration. In the following report, we highlight the considerable progress made in this fourth year including: 2 peer-reviewed publications, 3 submitted manuscripts that are in the review process, 7 presentations at the 2013 ARVO annual meeting, and 7 abstracts submitted to the 2014 ARVO meeting.

BODY

Personnel: Because of the funding situation, we had to cut back on the overall number of scientists working on this project. This past year, we had 3 PIs (Clark, Yorio, and Krishnamoorthy), 2 postdoctoral fellows (Kim and Liu), 3 technicians (Neubauer, Tebow, and Beckwith), and 5 graduate students working on various aspects of the VISION project. Please note that in some cases, salary support came from other funding sources.

CORE Facilities

We routinely run two acute models of ocular injury: optic nerve crush (ONC) and retinal ischemia/reperfusion injury (I/R). The chronic IOP elevation model is not routinely used and will reserved for evaluation of our best neuroprotection strategy. We now routinely and noninvasively evaluate retinal morphology using spectral domain-optical coherence tomography (SD-OCT) and retinal function using electroretinography (ERG) in both the ONC model (Liu et al., submitted for publication) and the I/R model (Kim et al. 2013). We have a dedicated histology core facility and use histological assessments at the 28 day endpoint for both models to quantify retinal ganglion cell counts, thicknesses of the various retinal layers, and neuron number and volume in the superior colliculus (visual center of the brain innervated by retinal ganglion cells). We also routinely evaluate gene

and protein expression changes using qPCR, western immunoblotting, and immunohistochemistry. In some cases, we use retinal ganglion cell cultures to evaluate neuroprotective mechanisms of action.

Neuroprotection Studies

Sigma-1 receptor: Activation of the sigma-1 receptor with selective agonists attenuates calcium influx mediated by L-type voltage gated calcium channels in cultured RGCs (Mueller et al. 2013). Sigma-1 receptor agonists also protect cultured primary RGCs from oxygen glucose deprivation (an in vitro form of ischemia) by activation of the ERK1/2 signaling pathway (Mueller et al, submitted for publication). We currently are testing wild type (normal) mice and sigma-1 receptor knockout mice in the retinal I/R injury model to see if the absence of sigma-1 receptor expression exacerbates retinal damage. In addition, we are testing the potential neuroprotective effects of systemic delivery of the sigma-1 receptor agonist pentazocine in the retinal I/R model.

JNKi: We have conclusively demonstrated that daily administration of the c-Jun N-terminal kinase (JNK) inhibitor SP600125 fully protected the retina from morphologic and functional damage in the mouse retinal I/R model. This therapy also protected the superior colliculus in the brain from I/R injury. This work was presented as a platform presentation at the 2013 ARVO annual meeting (by BJ Kim), and the manuscript describing our findings will be submitted for publication in the near future.

Brn3b: Brn3b is a transcription factor expressed in most retinal ganglion cells. Gene therapy by intravitreal injection of a AAV2.Br3b vector protected retinal ganglion cells from pressure-induced damage in a rat model of glaucoma. Brn3b also induced optic nerve regeneration in this model (Stankowska et al. 2013 ARVO Meeting and Krishnamoorthy et al. submitted to 2014 ARVO Meeting) and stimulated neurite outgrowth in cultured neurons (Phatak et al. 2013 ARVO Meeting).

AAV2.Nrn1: We found that expression of the retinal gene Neuritin1 (Nrn1) was downregulated in the retina and optic nerve shortly following optic nerve crush (ONC) injury (Sharma et al. 2014; Sharma et al. 2013 ARVO Meeting). We generated a gene therapy vector (AAV2.Nrn1) that was injected intravitreally into mouse eyes. The vector transduced retinal ganglion cells and provided robust Nrn1 expression for more than 6 weeks post-injection. Nrn1 gene therapy significantly protected the retina from ONC injury assessed by cell counts in the RGC layer and provided total ERG functional protection (Sharma et al. 2014; Sharma et al. submitted to 2014 ARVO Meeting). This novel gene therapy will also be tested in the retinal I/R model.

Complement: The complement system plays an important role in innate and acquired immunity, but also is involved in regulation of neuronal synaptic plasticity. We have recently shown that expression of the complement component C1q is elevated in the inner retina and superior colliculus shortly after ischemia/reperfusion injury, and this is strongly correlated with the activation of gliosis in both tissues (Silverman et al. 2013 ARVO Meeting). We are now performing in situ hybridization studies to determine which cell types in the retina and superior colliculus are generating C1q after I/R injury. In addition, we recently acquired C1q knockout mice from Simon John's laboratory at JAX, which will be used to determine whether C1q deficiency protects the inner retina and superior colliculus from I/R injury.

ERAD/UPR: Endoplasmic reticulum associated degradation (ERAD) and the unfolded protein response (UPR) are stress activated pathways that attempt to protect cells from a wide variety of insults. These pathways are activated in the retina after ONC and retinal I/R injury. However, chronic activation of these pathways can induce apoptotic death signals in overly stressed cells. We used mice deficient in several genes involved in ERAD/UPR induced apoptosis to determine whether these genes are involved in acute retinal injury. Deficiency in CHOP protected the inner retina structure and function in mice exposed to retinal I/R injury (Nashine et al. abstract submitted to 2014 ARVO meeting). Deficiency in Caspase 7 protected retinal ganglion cell structure and function after ONC injury (Chaoudhury et al. abstract submitted to 2014 ARVO meeting). In addition, we used gene therapy to deliver the ERAD/UPR chaperone BiP to retinal ganglion cells, which provided both structural and function protection from ONC injury (Liu et al. abstract submitted to 2014 ARVO meeting).

Key Research Accomplishments

- 2 published peer-reviewed manuscripts and 3 additional manuscripts that have been revised and resubmitted for publication
- 7 presentations at the 2013 ARVO meeting and 7 submitted abstracts for 2014 ARVO annual meeting
- Further characterized both acute models of injury to the visual axis in mice (1 manuscript published and one in review)
- Demonstrated statistically significant neuroprotection with 7 strategies
 - JNK inhibitor provided total structural and functional protection in ONC model
 - Sigma-1 receptor agonists attenuated calcium influx and protected cultured retinal ganglion cells from in vitro ischemia
 - Gene therapy with Brn3b protected retinal ganglion cells and promoted optic nerve regeneration in rodent model of glaucoma
 - Gene therapy with Neuritin1 structurally and functionally protected the retina in ONC model
 - CHOP knockout mice were structurally and functionally protected from retinal ischemia/reperfusion injury
 - Caspase 7 knockout mice were structurally and functionally protected the retina from ONC injury
 - Gene therapy with BiP structurally and functionally protected the retina from ONC injury
- Follow-up studies will be conducted with these lead neuroprotection strategies in the final year of this project

Conclusion

We have made significant progress on our overall research goals to identify new neuroprotective strategies to treat injury to the visual axis in three different models of ocular injury. All seven of these neuroprotective strategies provided both structural and functional protection to retinal neurons. This significant progress was made despite receiving only two years of funding for this ambitious five year project.

REPORTABLE OUTCOMES

Manuscripts

Kim, BJ, Braun TA, Wordinger RJ, Clark AF. Progressive morphological changes and impaired retinal function associated with temporal regulation of gene expression after retinal ischemia/reperfusion injury in mice. *Molecular Neurodegeneration* 8:21, 2013.

Mueller BH, Park Y, Daudt DR, Ma H-Y, Akopova I, Stankowska D, Clark AF, Yorio T. Sigma-1 receptor stimulation attenuates calcium influx through activated L-type voltage-gated calcium channels in primary retinal ganglion cells. *Experimental Eye Research* 107:21-31, 2013.

Mueller BH, Park Y, Ma H-Y, Dibas A, Clark AF, Yorio T. Sigma-1 receptor stimulation protects retinal ganglion cells from ischemic-like insult through the activation of extracellular-signal-related kinases 1/2. Submitted for publication.

Liu Y, McDowell CM, Zhang Z, Tebow HE, Wordinger RJ, Clark AF. Monitoring retinal morphological and functional changes in mice following optic nerve crush. Resubmitted for publication (revised manuscript being reviewed)

Sharma TP, McDowell CM, Liu Y, Wagner AH, Thole D, Faga B, Wordinger RJ, Braun TA, Clark AF. Optic nerve crush induces spatial and temporal gene expression patterns in retina and optic nerve of BALB/cJ mice. Resubmitted for publication (revised manuscript being reviewed)

Abstracts

Silverman S, McDowell CM, Kim B-J, Wordinger RJ, Clark AF. Complement and glial activity in the retinocollicular pathway of mice in a novel model of glaucoma. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 421.

Liu Y, Zhang Z, Wordinger RJ, Libby RT, Pang I-H, Clark AF. Differential activation of p38 and c-jun N-terminal kinase in the visual pathway following optic nerve crush. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 422.

Putliwala T, Liu Y, Wordinger RJ, Clark AF. Role of neuritin 1 in response to optic nerve crush. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 431.

Kim BJ, Liu Y, Silverman S, Wordinger RJ, Libby RT, Pang I-H, Clark AF. Protective effects of JNK inhibition in retinal ganglion cells and in retinal ischemia/reperfusion injury. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2624.

Mueller BH, Park YH, Ma H-Y, Yorio T. Sigma-1 receptor stimulation protects purified RGCs from ischemic insult through the phosphorylation of extracellular signal kinase 1/2. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 3699.

Stankowska DL, Minton AZ, Krishnamoorthy RR. Brn3b mediated regeneration of the optic nerve in a rodent model of glaucoma. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 6357.

Phatak N, Stankowska DL, Krishnamoorthy RR. Overexpression of the POU domain transcription factor, Brn3b, causes neurite outgrowth in cultured PC12 cells. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 6364.

Nashine SR, Kim BJ, Clark AF, Pang I-H. Role of C/EBP homologous protein (CHOP) in the survival of retinal ganglion cells after retinal ischemia/reperfusion injury. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 1729

Liu Y, Sharma TP, Wordinger RJ, Gorbatyuk MS, Clark AF. Gene delivery of GRP78/BiP promotes retinal ganglion cell survival following optic nerve crush. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2426

Choudhury S, Liu Y, Clark AF, Pang I-H. Knockout Of Caspase-7 Protects Against Optic Nerve Crush-Induced Retinal Ganglion Cell Death. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2667

Sharma TP, Liu Y, Pang I-H, Wordinger RJ, Clark AF. Neuritin1 Promotes Retinal Ganglion Cell Survival And Axonal Regeneration Following Optic Nerve Crush. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2669.

Mueller BH, Park YH, Ma H-Y, Yorio T. Prolonged NMDA stimulation induces neuroprotective pathways and enhances survivability of primary retinal ganglion cells. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 1903

Park YH, Mueller BH, McGrady N, Dibas A, Yorio T. Retinal ganglion cells are resistant to AMPA receptor mediated excitotoxicity. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 1904

Krishnamoorthy RR, Stankowska DL. Brn3b mediated axonal regeneration of the optic nerve in a rodent model of glaucoma. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2183

REFERENCES: None

APPENDICES: None